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INTERACTION OF ACRIDINES WITH DNA

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It is known that two types of bonding exist between acridines and DNA in its native double helical configuration [1-4]. The first type of bonding is observed at small ratios of the number of acridine molecules to the number of DNA nucleotides ($P/D \geq 4$, where P - the number of nucleotides, D - the number of acridine molecules), the second is observed at large concentrations of molecules (acridine $P/D \leq 3$). We will limit ourselves to an examination of a complex of the first type.

Lerman [5] proposed that the first type of bonding corresponds to the "sandwiching" of acridine molecules between pairs of DNA bases. Such a "sandwiching" of necessity is accompanied by the rotation of a large quantity of various atomic groups of the phosphate shell around axes, lying, as a rule, in various planes. There is little foundation to assume that all these turns are not connected with overcoming large potential barriers and do not require large expenditures of energy. Since, however, the model of intercalation stems from such assumptions and thereby occupies an exceptional place in stereochemistry, the utilization of such a model for the DNA-acridine complex requires a more thorough foundation. This is also necessary because of the detection of the capability to interact, by an analogous method, on the part of non-flat aromatic analogs of acridine or molecules with large side chains [6]. Therefore there is interest in the construction of an alternative model,

not stemming a priori from the assumption that the native configuration of DNA is destroyed. For resolving this problem it is natural to examine possible types of binding of acridines on models.

Stereochemistry of the DNA-acridine complex. In the formation of complexes of acridines with DNA an important role is played by the electrostatic forces of the interaction between the cations of acridine and the phosphate groups of DNA, which is supported by the dependency of the quantity of bound molecules on ionic strength [1, 2--4].

As is known, the positive charge of the acridine molecules is concentrated on the ring nitrogen N_{10} (figure 1). Various substituents may significantly change the distribution of electron density in the molecule and the value of the charge on the N_{10} nitrogen. In accordance with [7] and the results of quantum-mechanical calculation [8], this holds true especially for molecules in which amino- or dimethylamino groups are found in the 2, 3 or 8 positions of the acridine ring. These molecules are characterized by the existence of resonance structures, in which the charge is not concentrated on the N_{10} nitrogen, but on the nitrogens of the amino groups, which is confirmed by the extremely low basicity of the amino groups in these positions. [7].

It is apparent that in a complex of acridines with DNA, the atomic groups of the acridines, bearing a positive charge, are found as close as possible to the phosphate groups. From a stereochemical point of view it is most probable that the molecules of acridine are disposed in a narrow groove. In this case, in addition to the electrostatic interaction with phosphate groups, the molecules of acridine may take part in a van der Waals interaction with bases and other atomic groups of DNA.

For a detailed description of the complex we will compare each nucleotide with a specific index, characterizing its place in the polynucleotide chain, and propose that it increases by a unit upon transition from the C_3' atom of ribose of one nucleotide to the C_5' atom of ribose of the other. This makes it possible to number the nucleotides in one of the polynucleotide chains. In order to determine the indices of nucleotides in the other chain we will assume that the nucleotides of the two chains, bound with each other by an axis of symmetry of the second order, possess identical indices. If a molecule of proflavine (2-8 diaminoacridine) is placed in the narrow groove so that the ring nitrogen N_{10} is found half way between the oxygen O_{III} of the i phosphate group of the first chain and the oxygen O_{III} of the i + 3 phosphate group of the second chain, then here one of the amino groups of proflavine turns out to be located next to the oxygen O_{III} of the i + 1 phosphate group of the first chain, and the other - next to the oxygen O_{III} of the i + 2 phosphate of the second chain (figure 2). We conditionally designate one of the polynucleotide chains as the first. Kurnick [9] put forth the proposal on the importance of the congruence of the distance between the amino groups and the phosphate groups of DNA. The distance between the centers of gravity of the i and the j O_{III} - atoms of the two polynucleotide chains is expressed in cylindrical coordinates in the following manner $d_{ij} = \sqrt{2r^2[1-\cos(\varphi_i - \varphi_j)] + (z_i - z_j)^2}$, where r - the radial distance of O_{III} atoms from the axis of the helix; φ_i, φ_j - the azimuth angle of the i and j atoms correspondingly; z_i, z_j - the Z-coordinates of these atoms (designations of atoms and the system of coordinates are the same as in the paper [10]). Using the coordinates of the DNA atoms for model III of Langridge et al. [10] we find:

$d_{i,i+3} \approx 12.4$ Å, $d_{i,i+2} \approx 11.2$ Å, $d_{i+1,i+2} \approx 13.5$ Å.

If for the acridine ring we use the values for the lengths of the bonds and the valency angles from [11], and for the amino groups of proflavine we accept the values presented in [12] for aniline, then the distance between the centers of gravity of the amino groups in a molecule of proflavine turns out to be equal to 9.4 Å. The van der Waal radii for oxygen and nitrogen are correspondingly 1.4 Å and 1.5 Å. Therefore the change in the orientation of the i + 1 and the i + 2 phosphate groups of the two chains, at which the O_{III} oxygens are shifted to the side of the narrow groove, makes it possible for the nitrogens of the proflavine amino groups to be in van der Waal contact with the O_{III} oxygens of the two chains. X-ray data are little sensitive to small turns of a phosphate group around its center [10]. We propose that these oxygens bear a single negative charge in a neutral medium. Probably the electrostatic field of these charges may induce in the molecule of proflavine that distribution of electronic density which is presented schematically in figure 1. A characteristic feature of it is that there is an increase in the share of resonance structures with a localization of the double bond between the carbon ring and the nitrogen of the amino groups. This is particularly manifested in the fact that the positive charge on the N_{10} nitrogen is decreased, but on the nitrogens of the amino groups it is increased. Apparently the redistribution of electronic density makes it possible to explain the reduction in the reaction capability of amino groups of the bound proflavine in respect to nitrous acid [13]. Another confirmation of such a redistribution of electronic density is the shift of the pH of the bound acridine orange into the area of more alkaline pH values (ring nitrogen N_{10} is a titrate group) [14]. It probably is also

responsible for the long-wave shift of the adsorption spectrum for the complex of proflavine with DNA [1], since the overall length of the combined system of bonds is increased. This change of optical properties can be viewed as an obvious result of the Londonov interaction between the molecule of acridine and the bases of the DNA. It is evident that it cannot be an argument in favor of a model of intercalation, since analogous spectral changes are observed during complexing with DNA molecules, the inner building of which is excluded [15, 16]. Moreover, such an interpretation encounters difficulty, since the absorption spectra do not undergo any changes at such pH values, when various groups of bases are titrated [14].

Acridines, in which there are no substituents in the 2 and 8 positions of the acridine ring, may have a somewhat different orientation in the narrow groove. Since $d_{i,i+2} < d_{i,i+3}$, then it is more probable that the molecule is disposed in such a way that the N_{10} nitrogen is found between the O_{III} oxygens of the i and the i + 2 phosphate groups. We propose that both these oxygens carry a negative charge, an excess of which can always be neutralized by Na^+ cations, and the free space remaining in the narrow groove is filled with water.

The aromatic ring of the acridine molecule is "submerged" in the narrow groove and its interaction with the bases and other atomic groups apparently bears mainly a van der Waal nature. In certain cases the conditions for such an interaction are particularly favorable. For example, the methyl groups in the 3 and 7 positions in acridine yellow are found in contact with aliphatic groups of ribose, and the amino group in the 5 position in the molecule of 2-5 diaminoacridine probably interacts with the formation of a hydrogen bond with the carbonyl group of thymine. This may correlate with the strong mutagenic action of such compounds [17]. The mechanism of mutagenesis through acridines

is probably connected with the mechanism of genetic recombinations, [17, 18]. The circumstance that acridines form "clamps" between the i and the i + 1 or the i and the i + 2 nucleotides of the two chains may lead to an increase in the frequency of uneven crossing over with the deletion or insertion of one pair of bases [18].

It may also be responsible for the increase in the melting temperature of DNA during complexing with acridine orange [15, 19].

In the described complex one molecule of proflavine falls on 4 phosphate groups, which explains the limiting ratio of the number of bound molecules to the number of nucleotides. For the "strong" binding of proflavine it equals 1/4 [1, 6]. Apparently this limiting ratio should be in all acridines, though its value may differ somewhat from 1/4 [6].

It can also be expected that acridines decrease the flexibility of the DNA molecule and thanks to this increase the viscosity of the DNA solutions [5]. The energetics of binding of acridines also does not contradict the proposed model. It has been shown that for a molecule of proflavine at room temperature enthalpy of binding comprises - 5.2 kcal/mole, and entropy + 7.5 cal/mole • degrees, that is, the binding of proflavine is accompanied by a considerable increase of entropy [20]. Apparently this increase is the result of the displacement by proflavine of a significant quantity of water molecules, found in the narrow ~~furrow~~ groove. The breakdown of the hydrate membrane of proflavine during its binding with DNA could lead to a certain increase of entropy. This apparently is an unique possible explanation for the model of intercalation. The plane of the aromatic ring of acridine forms a certain angle with the plane of the bases. Its value may be diverse for different acridines, but it is always found within the limits of $0° < \theta < 30°$, which does not contradict data based on the measuring of dichromism in a stream and polarization of fluorescence [21].

It was also demonstrated that a molecule of actinomycin is not formed between the pair of DNA bases, since the plane of its aromatic ring forms an angle of $23^{\circ} \pm 5^{\circ}$ with the plane of the bases [13]. At the same time there is a basis to propose that the mechanism of binding actinomycin and acridines is the same [15, 22]. The proposed model can probably be extended to certain other dyes -- methylene blue, safranine, etc.

Diffraction of X-rays on fibers of DNA-proflavine.

Lerman [5] detected that a 3.4 Å meridional reflexion, corresponding to the axial projection of the pair of bases, is retained on the diffraction pictures from oriented gels of DNA. However, the characteristic distribution of the reflexions on layer lines, corresponding to the DNA configuration in the B-form, disappears. Lerman interpreted this change of diffraction pictures on the basis of the model of intercalation. However, such an interpretation cannot be considered as conclusive, since such a change of diffraction pictures may be caused by a number of reasons. In particular, a deterioration in the packing of the DNA molecules in the fiber, without a change of their configuration, leads to the disappearance of the acute reflexions on many layer lines [23]. A certain change in the configuration of the phosphate frame does not contradict the proposed model. Thus the distance between the i + 1 and i + 2 oxygens O_{III} of the two chains decreases from 13.5 Å to $\sim$12 Å. A certain change should also be expected in the coordinates of the atoms of the i and i + 3 nucleotides. If some of these base pairs merge in the plane, perpendicular to the axis of the molecule, then the change in the configuration of the shell may be more significant. In accordance with the proposed model the interaction with proflavine is accompanied by the displacement of a significant amount of water molecules from the narrow groove and the formation of vacuums in it. It is also possible that

this provides a certain contribution in the diffraction from wet gels.

Scattering of X-rays at small angles. Luzzati, Lerman and Mason [24] detected that in solution the DNA-proflavine complex scatters X-rays like a rod-like particle, the mass per unit of length and radius of inertia of which is less than the mass per unit of length and radius of inertia of the DNA itself. The authors suggest that this result, and also the fact of the retention of the 3.4 Å meridional reflexion in DNA-proflavine gels, excludes any "external" joining of the proflavine molecule. Therefore we will examine this experiment in more detail.

If there is a solution of rod-like particles and $\varrho(x,y,z)$ - is the function describing the distribution of electronic density in such a particle, and ϱ_0 - is the electronic density of the solvent, then in accordance with the Babine principle such a solution will scatter X-rays, since particles with an electron density $\varrho_1 = \varrho(x,y,z) - \varrho_0$, found in the vacuum, scatter.

Luzzati, Nicolaeff and Mason showed that in actuality at a low ionic strength solutions of DNA scatter X-rays as rod-like particles, the mass per unit length and radius of inertia of which correspond to the Watson-Crick model [25]. This means that the electron density of the solvent in the vicinity of the DNA molecule, for example, in its grooves, does not differ strongly from the electron density of the solvent filling up the inter-molecule spaces. The interaction of proflavine with DNA may lead to a change in the average electron density in the grooves of DNA.

For the majority of a priori models possible of "external" joining of proflavine to DNA, the amplitude of scattering in the solution equals the amplitude of scattering from the DNA-proflavine complex in the vacuum minus the amplitude of scattering from volumes of DNA and proflavine filled with solvent.

However, if the molecules of proflavine are situated in the narrow groove, the latter statement ceases to be true. A molecule of proflavine inevitably displaces from the narrow groove a considerably greater number of water molecules than this would be expected from the value of the molecular volume of proflavine. Therefore even with small concentrations of proflavine the average electron density of the substance in the narrow groove differs from the electron density of the solvent. Scattering from the DNA-proflavine complex can be described, having assumed that it is characterized by a large value for the scattering volume

$$v_2 = v_1 + v \qquad (1)$$

where v_2 - is the scattering volume of the DNA-proflavine complex, v_1 - the volume of DNA (non-hydrated), v - the volume of the narrow groove.

We will assume that the volumes v_2, v_1 and v are calculated for one pair of bases. The specific volume of the DNA-proflavine complex is

$$\psi_2 = \frac{v_1 + v}{\bar{\rho}_1 v_1 + \bar{\rho} v}, \qquad (2)$$

where $\bar{\rho}_1$ - is the average electron density of DNA, $\bar{\rho}$ - the average electron density of the substance in the narrow groove.

In the first approximation

$$\bar{\rho} = \rho_0 - \partial \varkappa \nu, \qquad (3)$$

where ν - is the ratio of the number of moles of bound proflavine to the number of moles of the base pairs, $\varkappa$ - the positive or negative number, not depending on ν.

We will proceed from the assumption that the mass per unit length of the DNA itself when complexed with proflavine changes insignificantly.

Such an assumption is natural for this model, since during complexing the 3.4 Å meridional reflexion is retained [5]. This makes it possible to obtain the following expression for the mass per unit volume of the DNA-proflavine complex

$$\mu_2 = \mu_1 + \frac{\rho_0 - 2\chi\nu}{h}\upsilon, \qquad (4)$$

μ_1 - the mass per unit volume of DNA (non-hydrated), h = 3.36 Å.

The intensity of scattering from a solution of rod-like particles [26, 28] is

$$i_n(s) = (1-\rho_0\psi)^2 c_e \mu \frac{1-2\pi^2 R_e^2 s^2}{2s} + \ldots,$$

For slotted collimation [26]

$$J_n(s) = A\left[\tfrac{1}{2}\exp(-\pi^2 R^2 s^2) K_0(\pi^2 R_e^2 s^2)\right],$$

where

$$A = \mu c_e (1-\rho_0\psi)^2 \qquad (5)$$

R_e - the radius of inertia of the particle

$$s = \frac{2\sin\theta}{\lambda},$$

c_e - the concentration of scattering particles, expressed by the ratio of the number of electrons for the particle to the number of electrons for the solution.

For the DNA-proflavine complex

$$c_{e_2} = c_{e_1} \frac{\bar{\rho}_1\upsilon_1 + \rho_0\upsilon - 2\chi\nu\upsilon}{\bar{\rho}_1\upsilon_1(1+c_{e_1}\alpha\beta)} \qquad (6)$$

c_{e_1} - concentration of DNA; α - ratio of the number of electrons for a molecule of proflavine to the number of electrons for the pair of bases, α = 0.324; β - ratio of the number of moles for proflavine to the number of moles for the base pairs.

From (2), (3), (4) and (6) we find

$$\left(\frac{A}{c_{c_1}}\right)^{1/2} = \frac{(\bar{\rho}_1 - \rho_0)v_1 - 2\varkappa v}{\sqrt{3.36\bar{\rho}_1 v_1 (1 + c_{c_1} a\beta)}} \qquad (7)$$

Since $c_{c_1} a\beta \ll 1$

$$\left(\frac{A}{c_{c_1}}\right)^{1/2} = \frac{(\bar{\rho}_1 - \rho_0)v_1 - 2\varkappa v}{\sqrt{3.36\bar{\rho}_1 v_1}} \qquad (8)$$

Using experimental values for the meaning of $\left(\frac{A}{c_{c_1}}\right)$ for various β, it is possible to construct the dependency $\left(\frac{A}{c_{c_1}}\right)^{1/2}$ on β.

The resulting experimental curve was processed by the method of the least squares. It turned out that the dependency of $\left(\frac{A}{c_{c_1}}\right)^{1/2}$ on β is linear (figure 3), and is satisfactorily written by the equation

$$\left(\frac{A}{c_{c_1}}\right)^{1/2} = 1.25\beta + 3.90 \qquad (9)$$

In comparing the experimental curve with the dependency (8) in the assumption that $\nu = \beta$, we find $2\varkappa v = 40.6$.

The volume of the narrow groove v, limited by the planes $Z = 0$, $Z = 3.36$ Å, perpendicular to the axis of the molecule, and the cylindrical surface, passing through the centers of gravity of the phosphate groups, is approximately 300 Å^3 for the Langridge model III and others. From here $\varkappa \approx 0.7 \cdot 10^{-3}$.

We now evaluate the average density of the substance in the narrow groove when it is filled with proflavine. In the narrow groove a molecule of proflavine occupies the space corresponding to two pairs of bases. An examination on Kurto models shows that in this volume in the presence of a molecule of proflavine it is possible to find no more than 3--4 molecules of water. The average density in the narrow groove when it is most densely filled with proflavine is

$$\rho^* = \frac{C+m}{2v}, \qquad (10)$$

where

C - the number of electrons of the proflavine molecule,

m - the total number of electrons of the water molecules which remain in the volume $2v$ after the fitting of the proflavine molecule

$$\rho^* = \frac{110+3\cdot 10}{600} \approx 0.23\, e/\text{Å}^3,$$

from here $\varkappa = 0.1\, e/\text{Å}^3$.

This evaluation shows that the average electron density of the substance in the narrow groove when it is filled with proflavine is less than the average density of the solvent. Moreover, with the extremely rough assu mptions made its quantitative value is found in reasonable accordance with the experiment.

We note that for other models of "external" combining of proflavine the value of $\left(\frac{A}{C_{c_1}}\right)^{\frac{1}{2}}$ increases with development of β (it is assumed that the configuration of DNA is not changed). It is known that several types of binding of proflavine with DNA exist [4, 8].

If this takes place under experimental conditions, then the observed value of $\left|\frac{d\left(\frac{A}{C_{c_1}}\right)^{\frac{1}{2}}}{d\beta}\right|$ is lowered.

In this manner it is possible to explain the lessening of the radius of inertia. In truth, for the DNA-proflavine complex

$$R^2_{C_2} = \frac{\iiint_{v_1}(x^2+y^2)[\rho_1(x,y,z)-\rho_0]\,dv_1 + \iiint_{v}(x^2+y^2)[\rho(x,y,z)-\rho_0]\,dv}{\iiint_{v_1}[\rho_1(x,y,z)-\rho_0]\,dv_1 + \iiint_{v}[\rho(x,y,z)-\rho_0]\,dv}$$

or

$$R^2_{C_2} = \frac{R^2_{C_1}(\overline{\rho_1}v_1 - \rho_0 v_1) + (\overline{\rho}-\rho_0)\,v\,\overline{[x^2+y^2]_v}}{\overline{\rho_1}v_1 - \rho_0 v_1 + \overline{\rho}v - \rho_0 v}. \qquad (11)$$

Making use of (3) we find:

$$R^2_{C_2} = \frac{R^2_{C_1}(\overline{\rho_1}v_1 - \rho_0 v_1) - 2\varkappa v\,\overline{[x^2+y^2]_v}}{\overline{\rho_1}v_1 - \rho_0 v_1 - 2\varkappa v \cdot v}, \qquad (12)$$

where R_{C_1} - is the radius of inertia of DNA (without the Na^+ ions), $\overline{[x^2+y^2]_v}$ - the average square of the distance from the axis for points, found in the space of the narrow groove (averaging based on the space of the narrow groove). Interpolating the designation $\delta = \frac{2\varkappa v}{\overline{\rho_1}v_1 - \rho_0 v_1}$, $\gamma = \frac{\nu}{1-\delta\nu}$ and disregarding the high powers $\left(1 - \frac{\overline{[x^2+y^2]_v}}{R^2_{C_1}}\right)\delta\cdot\gamma$,

we find

$$R_{C_2} \approx R_{C_1}\left[1 + \left(1 - \frac{\overline{[x^2+y^2]_v}}{R^2_{C_1}}\right)\frac{\delta\cdot\nu}{2}\right]. \qquad (13)$$

This dependency is obtained under the assumption that DNA, and not its sodium salt, interacts with proflavine. If $\nu \cdot N$ of proflavine molecules are bound with a molecule of DNA, having N pairs of bases, then they replace $\nu \cdot n$ of Na^+ ions. Taking this circumstance into consideration, for the average radius of inertia for the complex of DNA sodium salt with proflavine we obtain $R_{c_3} = R_c - \nu \cdot (R_c - R_{c_2})$, where R_c - is the radius of inertia of DNA Na^+ or

$$R_{c_2} = R_c - \nu \cdot (R_c - R_{c_1}) - \frac{R_{c_1}}{2}\left(\frac{\overline{[x^2+y^2]}_0}{R_{c_1}^2} - 1\right)\frac{\delta\nu^2}{1-\delta\nu}. \qquad (14) \qquad (14)$$

For the Langridge Model III and others [10] R_{c_1} = 6.95 Å, the experimental significance of the value R_c = 8.4 Å [25]. The value $\sqrt{\overline{[x^2+y^2]}_0} \approx 7.5$ Å. Making use of these values we find

$$R_{c_2} \approx R_c - 1{,}45\nu - 0{,}2\frac{\nu^2}{1-0{,}32\nu} \qquad (15)$$

The values of the radius of inertia, obtained from (15) under the assumption that $\nu = \beta$, are found in satisfactory agreement with the experimental values (see the table). The low angle scattering from a rod-like particle is established by the parameters A and R_c and therefore does not contradict the proposed model.

Luzzati et al. [25] detected an increase in the scattering capacity of DNA in solutions of NaCl and NaBr. It is logical to assume that the additional scattering capacity is characterized by a larger, in comparison with the surrounding solution, concentration of cations and a lesser concentration of anions. An analogous increase of scattering capacity apparently takes place for the cesium salt of DNA as a result of the great scattering capability of Cs^+. It is little

probable that DNA Cs in an isotropic solution has a configuration which is different from the Watson-Crick model [5]. Nevertheless the small angle scattering from gels of DNA Cs is found in good accordance with this model. [27] It is known that the small angle scattering in gels, in contrast to scattering in solutions, depends little on the value of the specific capacity of the particle, and therefore probably gives a more reliable value for the mass per unit length.

The interaction of acridines with DNA is distinguished from the interaction of metal cations with it by the fact that for acridines there exist additional limitations, connected with the packing of the massive organic cation. The narrow groove corresponds to that part of the surface of the DNA--cylinder, which is characterized by the maximum surface density of the charged phosphate groups. Therefore it is natural that there should be a primary disposition of cations, metals and acridines.

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β	Radius of gyration in Å		β	Radius of gyration in Å	
	Experimental values	Theoretical values		Experimental values	Theoretical values
0.000	8.4	8.4	0.304	7.8	8.0
0.097	8.2	8.3	0.324	7.8	7.9
0.103	8.2	8.3	0.348	7.7	7.9
0.202	8.0	8.1	0.420	7.6	7.8
0.208	8.0	8.1	0.456	7.5	7.7
			0.510	7.5	7.6

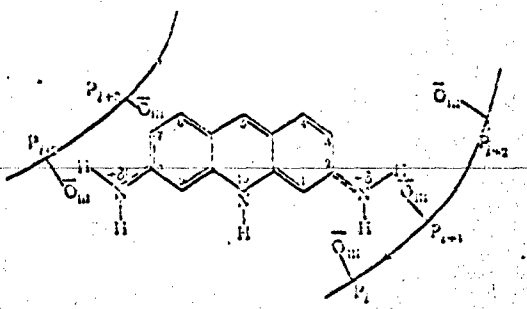

Figure 1. Schematic representation of the proflavine--DNA complex. Each line represents a separate polynucleotide chain, and the sequence of atoms in it is shown by the arrow.

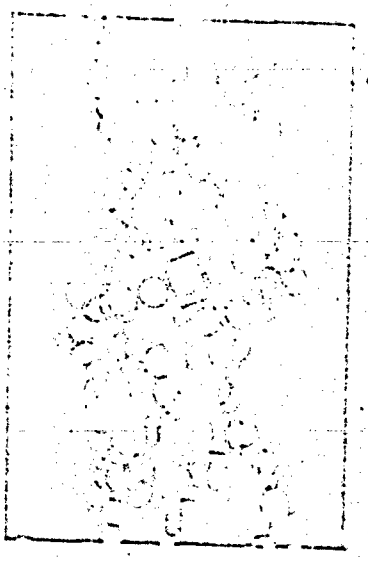

Figure 2. Three dimensional model of the proflavine--DNA complex.

Coordinates of the nitrogen atoms of proflavine amino groups

	r	φ	z
N_2	8 ± 0.5 Å	$52^\circ \pm 3^\circ$	3.3 ± 0.5 Å
N_8	8 ± 0.5 Å	$16^\circ \pm 3^\circ$	0 ± 0.5 Å

The possibility of the formation of a hydrogen bond with the amino groups is not excluded.

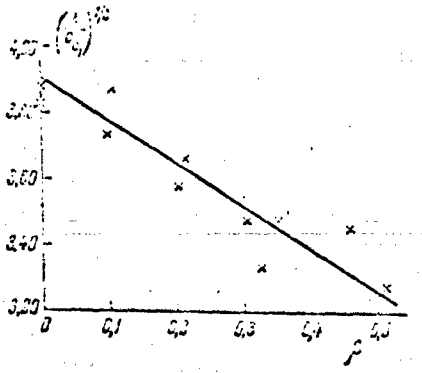

Figure 3. Dependency of the value $\left(\frac{A}{c_{e_i}}\right)^{1/2}$ on β.